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Modified Trichrome Staining Technique with a Xylene Substitute

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Hemo-De was evaluated and found to be a suitable replacement for xylene in the Wheatly trichrome staining of polyvinyl alcohol-fixed fecal smears used in the microscopic identification of intestinal protozoans.

Xylene is widely used in histology and cytology laboratories as a clearing agent. It is also used for a similar function in the trichrome staining technique of the polyvinyl alcohol (PVA)-fixed films made from fecal material derived from patients suspected of being infected with intestinal protozoans.

Because of the potential toxic and fire hazards of xylene, xylene substitutes have been introduced into some laboratories. These newer reagents are reportedly less hazardous than xylene in that they are nontoxic, nonflammable, and biodegradable, and they are classified by the Food and Drug Administration as GRAS (generally regarded as safe). One of these substitutes, Hemo-De (produced by PMP Medical Industries, Inc., Irving, Tex., and distributed by Fisher Scientific Co., Pittsburgh, Pa. [catalog no. 15-182-507A]) was selected for evaluation as a possible replacement for xylene in the trichrome staining technique.

PVA-fixed fecal material which was previously examined by the standard technique and found to contain the protozoans indicated below was selected to evaluate Hemo-De as a xylene substitute in the trichrome staining technique: *Entamoeba histolytica* cysts and trophozoites, *Entamoeba coli* cysts and trophozoites, *Entamoeba hartmanni* trophozoites, *Endolimax nana* cysts and trophozoites, *Giardia lamblia* cysts and trophozoites, *Dientamoeba fragilis* trophozoites, and *Iodamoeba butschlii* cysts and trophozoites. A duplicate set of slides was prepared from PVA-fixed material. One set of slides was stained by the standard trichrome technique of Wheatly (1), in which we allowed the slides to remain in the various solutions for the maximum times except for a brief exposure to the acidified 90% alcohol. The second set of slides was stained in five modifications of steps 8 and 9 of the standard trichrome technique (Table 1).

The standard staining technique and slides made from the same PVA-fixed fecal material served as controls for each modified staining technique used in this study. To eliminate possible bias, one of the authors (R.N.) stained and coded the slides while another (A.L.) read and evaluated each technique in terms of clarity, color differentiation, and physical appearance of the organisms.

Techniques A and B, which did not incorporate phenol in step 8 of the procedure, failed to adequately clear the preparation (Table 2). However, when Hemo-De replaced xylene in steps 8 and 9 of the standard procedure, as in technique C, clearing was accomplished, but the staining characteristics appeared diminished. When the reaction times were changed as in technique E, the clearing and staining characteristics observed were equivalent to or better than those seen in the control slides.

TABLE 1. Five modifications of steps 8 and 9 of the standard trichrome technique (1) used to evaluate the xylene substitute[a]

Technique	Substitute	Reaction time (min)
A	Hemo-De	20
	Step omitted	
B	Hemo-De	10
	Step omitted	
C	Carbol–Hemo-De[b]	10
	Hemo-De	10
D	Carbol–Hemo-De[b]	10
	Hemo-De	1 dip
E	Carbol–Hemo-De	5
	Hemo-De	5

[a] The standard reagents are carbol-xylene and xylene, for which the reaction time is 10 min (1).
[b] One volume of melted phenol crystals plus three volumes of Hemo-De.

Because of the overall superiority of technique E (Table 3) over the other modifications (A through D), an in-depth comparison of the standard trichrome technique with the modified (E) technique was performed. The results of this comparison revealed that (i) no additional distortion or shrinkage of the organisms was observed in the modified technique when compared with the standard technique; (ii) in general, the organisms appeared to be more deeply stained by the modified technique; (iii) one species, *E. hartmanni*, appeared to be cleared better in the modified technique; (iv) several species, *I. butschlii*, *E. nana*, and *D. fragilis*, demonstrated better contrast to the background with the modi-

TABLE 2. Evaluation of the five variations of the standard trichrome technique

Technique	Comment
A	Slides inadequately cleared
B	Slight haze on all slides
C	All slides appeared washed out or over-decolorized
D	Good differentiation of protozoans, slight pale staining
E	Generally equivalent to the staining characteristics seen on the control slides

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TABLE 3. Modified trichrome staining technique of PVA-fixed fecal smears

Step	
1. 70% Alcohol plus iodine[a]	20 min
2. 70% Alcohol	5 min
3. 70% Alcohol	5 min
4. Trichrome stain[a]	8 min
5. 90% Alcohol, acidified[a]	1 quick dip
6. 95% Alcohol	2–3 dips
7. 95% Alcohol	5 min
8. Carbol/Hemo-De[b]	5 min
9. Hemo-De	5 min
10. Mount with cover slip, using Permount (Fisher Scientific Co.) or other mounting medium	

[a] See reference 1.
[b] One volume of melted phenol crystals plus three volumes of Hemo-De.

fied technique; (v) the modified technique shortened the procedure; (vi) the modified technique eliminated the potential health hazards of xylene; and (vii) the modified technique replaced the irritating odor of xylene with the more pleasant citrus fragrance of Hemo-De.

When the advantages delineated above are considered, Hemo-De is a welcome substitute for xylene in the trichrome staining technique for intestinal protozoans. Use of this technique in parallel with the standard technique for several months confirmed the results reported herein.

LITERATURE CITED

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